

L Number	Hits	Search Text	DB	Time stamp
1	790	helper same probe	USPAT; US-PGPUB; DERWENT	2003/06/27 11:54
2	714	(helper same probe) and 18S	USPAT; US-PGPUB; DERWENT	2003/06/27 11:54
3	140	helper near4 probe	USPAT; US-PGPUB; DERWENT	2003/06/27 11:54
4	14	(helper near4 probe) and 18S	USPAT; US-PGPUB; DERWENT	2003/06/27 11:59
5	117	"5030557"	USPAT; US-PGPUB; DERWENT	2003/06/27 11:56
6	2	(helper near4 probe) and 18S and interacting	USPAT; US-PGPUB; DERWENT	2003/06/27 11:59
7	2	(helper near4 probe) and 18S and ribofuranosyl	USPAT; US-PGPUB; DERWENT	2003/06/27 11:59

=> d his

(FILE 'HOME' ENTERED AT 08:31:00 ON 27 JUN 2003)

FILE 'REGISTRY' ENTERED AT 08:31:13 ON 27 JUN 2003

L1 212 S CTATCAGCTTTAGACGGTAGGG/SQSN

L2 2 S L1 AND 23-100/SQL

FILE 'CAPLUS' ENTERED AT 08:32:49 ON 27 JUN 2003

L3 1 S L2

S CTATCAGCTTT/SQSN

FILE 'REGISTRY' ENTERED AT 08:34:34 ON 27 JUN 2003

L4 10736 S CTATCAGCTTT/SQSN

FILE 'CAPLUS' ENTERED AT 08:34:58 ON 27 JUN 2003

L5 1194 S L4

L6 0 S L5 AND 18-100/SQL

FILE 'REGISTRY' ENTERED AT 08:36:23 ON 27 JUN 2003

L7 10736 S CTATCAGCTTT/SQSN

L8 20 S L7 AND 18-100/SQL

FILE 'CAPLUS' ENTERED AT 08:37:37 ON 27 JUN 2003

L9 5 S L8

FILE 'REGISTRY' ENTERED AT 08:38:33 ON 27 JUN 2003

L10 1667 S AGACGGTAGGG/SQSN

L11 5 S L10 AND 18-100/SQL

FILE 'CAPLUS' ENTERED AT 08:39:27 ON 27 JUN 2003

L12 2 S L11

=>

```
=> s align?
L1      166900 ALIGN?

=> s parvum or crptosporidium
L2      9814 PARVUM OR CRPTOSPORIDIUM

=> s gondii or neurona or muris or gigantea or cruzi or capracanis or arieticansi
or tenella
L3      47809 GONDII OR NEURONA OR MURIS OR GIGANTEA OR CRUZI OR CAPRACANIS
OR ARIETICANSI OR TENELLA
```

```
=> s l2 and l3 and l1
L4      19 L2 AND L3 AND L1
```

```
=> dup reml 4
ENTER REMOVE, IDENTIFY, ONLY, OR (?) :end
```

```
=> dup rem l4
PROCESSING COMPLETED FOR L4
L5      13 DUP REM L4 (6 DUPLICATES REMOVED)
```

```
=> d ibib ab 1-13
```

```
L5      ANSWER 1 OF 13  CAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:      2003:356474  CAPLUS
DOCUMENT NUMBER:      138:362638
TITLE:      Diagnosis and treatment of infectious diseases through
indel-differentiated proteins
INVENTOR(S):      Reiner, Neil E.; Tcherkassov, Artem; Nandan, Devki
PATENT ASSIGNEE(S):      The University of British Columbia, Can.
SOURCE:      PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DOCUMENT TYPE:      Patent
LANGUAGE:      English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:
```

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003037926	A1	20030508	WO 2002-CA1689	20021101
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
PRIORITY APPLN. INFO.:			CA 2001-2360987	A 20011101
			US 2002-349339P	P 20020122
			US 2002-349371P	P 20020122
			US 2002-393385P	P 20020705
AB A compd. capable of specifically binding to pathogen EF-1.alpha. but not host EF-1.alpha., wherein the compd. binds to any part of an amino acid sequence having at least 70% sequence identity to amino acids 240-230 of SEQ ID NO:22 of EF-1.alpha. from Leishmania donovani.				
REFERENCE COUNT: 10			THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT	

```
L5      ANSWER 2 OF 13      MEDLINE      DUPLICATE 1
```

ACCESSION NUMBER: 2002312290 MEDLINE
 DOCUMENT NUMBER: 22048751 PubMed ID: 12054017
 TITLE: Molecular phylogeny and evolutionary relationships of
 Cryptosporidium parasites at the actin locus.
 AUTHOR: Sulaiman Irshad M; Lal Altaf A; Xiao Lihua
 CORPORATE SOURCE: Division of Parasitic Diseases, National Center for
 Infectious Diseases, Centers for Disease Control and
 Prevention, U.S. Department of Health and Human Services,
 Atlanta, Georgia 30341, USA.
 CONTRACT NUMBER: DW75937730-01-0
 SOURCE: JOURNAL OF PARASITOLOGY, (2002 Apr) 88 (2) 388-94.
 Journal code: 7803124. ISSN: 0022-3395.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200206
 ENTRY DATE: Entered STN: 20020611
 Last Updated on STN: 20020628
 Entered Medline: 20020627

AB To further validate previous observations in the taxonomy of
 Cryptosporidium parasites, the phylogenetic relationship was analyzed
 among various Cryptosporidium parasites at the actin locus. Nucleotide
 sequences of the actin gene were obtained from 9 putative Cryptosporidium
 species (*C. parvum*, *C. andersoni*, *C. baileyi*, *C. felis*, *C.*
meleagridis, *C. muris*, *C. saurophilum*, *C. serpentis*, and *C.*
wrairi) and various *C. parvum* genotypes. After multiple
alignment of the obtained actin sequences, genetic distances were
 measured, and phylogenetic trees were constructed. Results of the
 analysis confirmed the presence of genetically distinct species within
 Cryptosporidium and various distinct genotypes within *C. parvum*.
 The phylogenetic tree constructed on the basis of the actin sequences was
 largely in agreement with previous results based on small subunit rRNA,
 70-kDa heat shock protein, and Cryptosporidium oocyst wall protein genes.
 The Cryptosporidium species formed 2 major clades; isolates of *C.*
andersoni, *C. muris*, and *C. serpentis* formed the first major
 group, whereas isolates of all other species, as well as various *C.*
parvum genotypes, formed the second major group. Intragenotype
 variations were low or absent at this locus.

L5 ANSWER 3 OF 13 MEDLINE
 ACCESSION NUMBER: 2001261987 MEDLINE
 DOCUMENT NUMBER: 21215633 PubMed ID: 11318578
 TITLE: Myosin diversity in Apicomplexa.
 AUTHOR: Heintzelman M B; Schwartzman J D
 CORPORATE SOURCE: Department of Anatomy, Dartmouth Medical School, Hanover,
 New Hampshire 03755, USA.
 CONTRACT NUMBER: AI-34760 (NIAID)
 SOURCE: JOURNAL OF PARASITOLOGY, (2001 Apr) 87 (2) 429-32. Ref: 16
 Journal code: 7803124. ISSN: 0022-3395.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 General Review; (REVIEW)
 (REVIEW, TUTORIAL)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-AF006627; GENBANK-AF066028; GENBANK-AF105117;
 GENBANK-AF105118; GENBANK-AF221131; GENBANK-AF222716;
 GENBANK-AF222717; GENBANK-AF273845; GENBANK-AF273846;
 GENBANK-AF273847; GENBANK-AF273848; GENBANK-AF273849;
 GENBANK-AF273850; GENBANK-AF273851; GENBANK-AF273852;
 GENBANK-AF273853; GENBANK-AF273854; GENBANK-AF273855;
 GENBANK-AF273856; GENBANK-AF273857; GENBANK-AF273858;
 GENBANK-AF273859; GENBANK-AF273860; GENBANK-AF273861;

GENBANK-AF273862; GENBANK-AF273863; GENBANK-AF273864;
GENBANK-AF273865; GENBANK-AF273866; GENBANK-AF273867;
GENBANK-AF273868; GENBANK-AF273869; GENBANK-AF273870;
GENBANK-AF273871; GENBANK-AF273872; GENBANK-AF273873;
GENBANK-AF273874; GENBANK-Z11718; GENBANK-AF006626;
GENBANK-P08799; GENBANK-P10568; SWISSPROT

ENTRY MONTH: 200105

ENTRY DATE: Entered STN: 20010521
Last Updated on STN: 20010521
Entered Medline: 20010517

AB A polymerase chain reaction (PCR) screen was used to examine the diversity of myosins in 7 Apicomplexan parasites: *Toxoplasma gondii*, *Plasmodium falciparum*, *Neospora caninum*, *Eimeria tenella*, *Sarcocystis muris*, *Babesia bovis*, and *Cryptosporidium parvum*. Using degenerate PCR primers compatible with the majority of known myosin classes, putative myosin sequences were obtained from all of these species. All of the sequences obtained showed greatest similarity to previously identified apicomplexan myosins, suggesting that the diversity of myosins in these parasites is limited. Myosin classes that are known to be widespread across the phylogenetic spectrum, e.g., the myosins I, II, and V, were not seen in the Apicomplexa. Thus, like the plants, the Apicomplexa may have evolved their own unique cohort of myosins that are responsible for the myosin-driven cellular functions observed in these parasites.

L5 ANSWER 4 OF 13 MEDLINE

ACCESSION NUMBER: 2001094625 MEDLINE

DOCUMENT NUMBER: 21030969 PubMed ID: 11191899

TITLE: Morphologic, host specificity, and genetic characterization of a European *Cryptosporidium andersoni* isolate.

AUTHOR: Sreter T; Egyed Z; Szell Z; Kovacs G; Nikolausz M; Marialigeti K; Varga I

CORPORATE SOURCE: Department of Parasitology and Zoology, Faculty of Veterinary Science, Szent Istvan University, Budapest, Hungary.

SOURCE: JOURNAL OF PARASITOLOGY, (2000 Dec) 86 (6) 1244-9.
Journal code: 7803124. ISSN: 0022-3395.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200101

ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010125

AB This study was undertaken in order to characterize a *Cryptosporidium muris*-like parasite isolated from cattle in Hungary and to compare this strain with other *Cryptosporidium* species. To date, the large-type oocysts isolated from cattle were considered as *C. muris* described from several mammals. The size, form, and structure of the oocysts of the Hungarian strain were identical with those described by others from cattle. An apparent difference between the morphometric data of *C. muris*-like parasites isolated from cattle or other mammals was noted, which is similar in magnitude to the differences between *Cryptosporidium meleagridis* and *Cryptosporidium felis* or between *Cryptosporidium serpentis* and *Cryptosporidium baileyi*. The cross-transmission experiments confirmed the findings of others, as *C. muris*-like oocysts isolated from cattle fail to infect other mammals. The sequence of the variable region of small subunit (SSU) rRNA gene of the strain was 100% identical with that of the U.S. *Cryptosporidium andersoni* and *C. andersoni*-like isolates from cattle. The difference between the SSU rRNA sequence of bovine strains and *C. muris* is similar in magnitude to the differences between *C. meleagridis* and *Cryptosporidium parvum* anthroponotic genotype or

between *Cryptosporidium wrairi* and *C. parvum* zoonotic genotype. Our findings confirm that the *Cryptosporidium* species responsible for abomasal cryptosporidiosis and economic losses in the cattle industry should be considered a distinct species, *C. andersoni* Lindsay, Upton, Owens, Morgan, Mead, and Blagburn, 2000.

L5 ANSWER 5 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
 ACCESSION NUMBER: 1999:520451 BIOSIS
 DOCUMENT NUMBER: PREV199900520451
 TITLE: Cryptosporidium is more closely related to the gregarines than to coccidia as shown by phylogenetic analysis of apicomplexan parasites inferred using small-subunit ribosomal RNA gene sequences.
 AUTHOR(S): Carreno, Ramon A.; Martin, Donald S.; Barta, John R. (1)
 CORPORATE SOURCE: (1) Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, N1G 2W1 Canada
 SOURCE: Parasitology Research, (Nov., 1999) Vol. 85, No. 11, pp. 899-904.
 ISSN: 0932-0113.
 DOCUMENT TYPE: Article
 LANGUAGE: English
 SUMMARY LANGUAGE: English
 AB The phylogenetic placement of gregarine parasites (Apicomplexa: Gregarinasina) within the Apicomplexa was derived by comparison of small-subunit ribosomal RNA gene sequences. Gregarine sequences were obtained from *Gregarina niphandrodes* Clopton, Percival, and Janovy, 1991, and *Monocystis agilis* Stein, 1848 (*Eugregarinorida* Leger 1900), as well as from *Ophriocystis elektroscirrha* McLaughlin and Myers, 1970 (*Neogregarinorida* Grasse 1953). The sequences were aligned with several other gregarine and apicomplexan sequences from GenBank and the resulting data matrix analyzed by parsimony and maximum-likelihood methods. The gregarines form a monophyletic clade that is a sister group to *Cryptosporidium* spp. The gregarine/*Cryptosporidium* clade is separate from the other major apicomplexan clade containing the coccidia, adeleids, piroplasms, and haemosporinids. The trees indicate that the genus *Cryptosporidium* has a closer phylogenetic affinity with the gregarines than with the coccidia. These results do not support the present classification of the *Cryptosporidiidae* in the suborder *Eimerioidirina* Leger, 1911.

L5 ANSWER 6 OF 13 MEDLINE DUPLICATE 2
 ACCESSION NUMBER: 1999313018 MEDLINE
 DOCUMENT NUMBER: 99313018 PubMed ID: 10386447
 TITLE: Phylogenetic analysis of *Cryptosporidium* isolates from captive reptiles using 18S rDNA sequence data and random amplified polymorphic DNA analysis.
 AUTHOR: Morgan U M; Xiao L; Fayer R; Graczyk T K; Lal A A; Deplazes P; Thompson R C
 CORPORATE SOURCE: World Health Organisation Collaborating Centre for the Molecular Epidemiology of Parasitic Infections and State Agricultural Biotechnology Centre, Murdoch University, Western Australia, Australia.
 SOURCE: JOURNAL OF PARASITOLOGY, (1999 Jun) 85 (3) 525-30.
 Journal code: 7803124. ISSN: 0022-3395.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-AF108863; GENBANK-AF108864; GENBANK-AF108865; GENBANK-AF108866
 ENTRY MONTH: 199907
 ENTRY DATE: Entered STN: 19990714
 Last Updated on STN: 19990714
 Entered Medline: 19990701

AB Sequence alignment of a polymerase chain reaction-amplified 713-base pair region of the *Cryptosporidium* 18S rDNA gene was carried out on 15 captive reptile isolates from different geographic locations and compared to both *Cryptosporidium parvum* and *Cryptosporidium muris* isolates. Random amplified polymorphic DNA (RAPD) analysis was also performed on a smaller number of these samples. The data generated by both techniques were significantly correlated ($P < 0.002$), providing additional evidence to support the clonal population structure hypothesis for *Cryptosporidium*. Phylogenetic analysis of both 18S sequence information and RAPD analysis grouped the majority of reptile isolates together into 1 main group attributed to *Cryptosporidium serpentis*, which was genetically distinct but closely related to *C. muris*. A second genotype exhibited by 1 reptile isolate (S6) appeared to be intermediate between *C. serpentis* and *C. muris* but grouped most closely with *C. muris*, as it exhibited 99.15% similarity with *C. muris* and only 97.13% similarity with *C. serpentis*. The third genotype identified in 2 reptile isolates was a previously characterized 'mouse' genotype that grouped closely with bovine and human *C. parvum* isolates.

L5 ANSWER 7 OF 13 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1999:103927 CAPLUS

DOCUMENT NUMBER: 130:347910

TITLE: Sequence and PCR-RFLP analysis of the internal transcribed spacers of the rDNA repeat unit in isolates of *Cryptosporidium* from different hosts
AUTHOR(S): Morgan, U. M.; Deplazes, P.; Forbes, D. A.; Spano, F.; Hertzberg, H.; Sargent, K. D.; Elliot, A.; Thompson, R. C. A.

CORPORATE SOURCE: World Health Organization Collaborating Centre for the Molecular Epidemiology of Parasitic Infections and State Agricultural Biotechnology Centre, Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6150, Australia

SOURCE: Parasitology (1999), 118(1), 49-58

CODEN: PARAAE; ISSN: 0031-1820

PUBLISHER: Cambridge University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The *Cryptosporidium* ITS1, 5.cntdot.8S and ITS2 rDNA regions from a no. of *Cryptosporidium* isolates from different hosts and geog. areas were cloned and sequenced in order to investigate the extent of sequence heterogeneity between human and cattle-derived isolates from different geog. locations and also between isolates of *Cryptosporidium* from different hosts such as cats, pigs, mice and a koala. Calf-derived isolates from different continents were virtually identical as were human-derived isolates from the UK and Australia. Genetic differences between *Cryptosporidium* isolates were extensive and were in fact greater than the level of nucleotide divergence between *Toxoplasma gondii* and *Neospora caninum* rDNA sequences. Based on the sequence information derived from this study, PCR-RFLP of the ITS1 region was undertaken in order to directly amplify and genotype *Cryptosporidium* isolates from different hosts. This PCR-RFLP approach can now be used for mol. epidemiol. studies, circumventing the need for costly sequencing and allowing a wider range of genetically different isolates to be examd.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 8 OF 13 MEDLINE

ACCESSION NUMBER: 1998360211 MEDLINE

DOCUMENT NUMBER: 98360211 PubMed ID: 9695098

TITLE: Molecular characterization of *Cryptosporidium* from various hosts.

AUTHOR: Morgan U M; Sargent K D; Deplazes P; Forbes D A; Spano F;

Hertzberg H; Elliot A; Thompson R C
 CORPORATE SOURCE: World Health Organization Collaborating Centre for the
 Molecular Epidemiology of Parasitic Infections, Murdoch
 University, Australia.. morgan@numbat.murdoch.edu.au
 SOURCE: PARASITOLOGY, (1998 Jul) 117 (Pt 1) 31-7.
 Journal code: 0401121. ISSN: 0031-1820.
 PUB. COUNTRY: ENGLAND: United Kingdom
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-AF099666; GENBANK-AF099667; GENBANK-AF099668;
 GENBANK-AF099669; GENBANK-AF102766; GENBANK-AF102767;
 GENBANK-AF102768
 ENTRY MONTH: 199808
 ENTRY DATE: Entered STN: 19980903
 Last Updated on STN: 20000303
 Entered Medline: 19980827

AB A 298 bp region of the *Cryptosporidium parvum* 18S rDNA and a 390
 bp region of the acetyl-CoA synthetase gene were sequenced for a range of
 human and animal isolates of *Cryptosporidium* from different geographical
 areas. A distinct genotype is common to isolates from cattle, sheep and
 goats and also an alpaca from Peru and is referred to here as the
 'calf'-derived *Cryptosporidium* genotype. Another genotype of
 'human'-derived isolates also appears to be conserved amongst human
 isolates although humans are also susceptible to infection with the 'calf'
Cryptosporidium genotype. Mice and pigs carry genetically distinct
 genotypes of *Cryptosporidium*. Three snake isolates were also analysed, 2
 of which exhibited *C. muris* genotypes and the third snake
 isolate carried a distinct 'mouse' genotype.

L5 ANSWER 9 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
 ACCESSION NUMBER: 1998:75624 BIOSIS
 DOCUMENT NUMBER: PREV199800075624
 TITLE: Investigating phylogenetic relationships within the
 Apicomplexa using sequence data: The search for homology.
 AUTHOR(S): Barta, John R. (1)
 CORPORATE SOURCE: (1) Dep. Pathobiol., Univ. Guelph, Guelph, ON N1G 2W1
 Canada
 SOURCE: Methods (Orlando), (Oct., 1997) Vol. 13, No. 2, pp. 81-88.
 ISSN: 1046-2023.
 DOCUMENT TYPE: General Review
 LANGUAGE: English

AB Whether stated explicitly or not, all molecular studies that seek to infer
 "homologies" among sequences or that attempt to determine the
 "relatedness" of taxa based on sequence comparisons are evolutionary
 studies. The generation of a reliable evolutionary hypothesis based on
 molecular sequences is dependent almost exclusively on the ability to
 align sequences such that bases or amino acids in the same
 position of two sequences are positionally homologous (i.e., they share
 the same position in the gene under study). The selection of suitable
 gene targets (commonly 18S small subunit rRNA gene sequences in the
 Apicomplexa) and appropriate ingroup and outgroup taxa will affect the
 ability to align sequences unambiguously. Mathematically derived
 alignments based on local sequence similarity have been shown to
 be less reliable than alignments based on conserved secondary
 structures coupled with an analysis of compensatory base changes. Use of
 staggered sequence alignments through hypervariable regions of
 18S small subunit rRNA gene sequences in which subsets of taxa are
 aligned independently may permit inclusion of more of the primary
 sequences with an associated increase in information content in the data
 set. The use of these highly variable regions is critical for determining
 the branching order of closely related terminal taxa in the phylum
 Apicomplexa.

L5 ANSWER 10 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:534945 BIOSIS
DOCUMENT NUMBER: PREV199598549245
TITLE: Effects of sequence **alignment** on the phylogeny of
Sarcocystis deduced from 18S rDNA sequences.
AUTHOR(S): Ellis, John (1); Morrison, David
CORPORATE SOURCE: (1) Dep. Cell Mol. Biol., Univ. Technol. Sydney, Gore Hill,
NSW Australia
SOURCE: Parasitology Research, (1995) Vol. 81, No. 8, pp. 696-699.
ISSN: 0932-0113.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The family Sarcocystidae contains a wide variety of parasitic protozoa, some of which are important pathogens of livestock and humans. The taxonomic relationships between two of the genera in this family (*Toxoplasma* and *Sarcocystis*) have been debated for a number of years and remain controversial. Recent studies, from comparisons of 18S rDNA-sequence data, have suggested that *Sarcocystis* is paraphyletic, although a hypothesis supporting monophyly of *Sarcocystis* could not be rejected. The present study shows that the phylogenetically informative nucleotide positions within the 18S rDNA are primarily located in the regions that make up the helices in the secondary structure of the 18S rRNA. A phylogenetic analysis of 18S rDNA-sequence data **aligned** by secondary structure constraints, or a subset of the data corresponding to all nucleotides found in the helices, provide unambiguous evidence supporting monophyly of *Sarcocystis*.

L5 ANSWER 11 OF 13 MEDLINE
ACCESSION NUMBER: 95348882 MEDLINE
DOCUMENT NUMBER: 95348882 PubMed ID: 7623193
TITLE: Sequence analysis and comparison of ribosomal DNA from bovine *Neospora* to similar coccidial parasites.
AUTHOR: Marsh A E; Barr B C; Sverlow K; Ho M; Dubey J P; Conrad P A
CORPORATE SOURCE: Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis 95616, USA.
SOURCE: JOURNAL OF PARASITOLOGY, (1995 Aug) 81 (4) 530-5.
Journal code: 7803124. ISSN: 0022-3395.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U17345; GENBANK-U17346; GENBANK-U17347;
GENBANK-U17349; GENBANK-U25043; GENBANK-U25044
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950911
Last Updated on STN: 19950911
Entered Medline: 19950829

AB The nuclear small subunit ribosomal RNA (nss-rRNA) gene sequence of *Neospora* spp. isolated from cattle was analyzed and compared to the sequences from several closely related cyst-forming coccidial parasites. Double-stranded DNA sequencing of 5 bovine *Neospora* spp. isolates (BPA1-4), 2 *Neospora caninum* isolates (NC-1 and NC-3), and 3 *Toxoplasma gondii* isolates (RH, GT-1, CT-1) were performed and compared to each other, as well as to other sequences available in GenBank for the NC-1 isolate, *Sarcocystis muris*, and *Cryptosporidium parvum*. There were no nucleotide differences detected between the *Neospora* spp. isolates from cattle and dogs. Four nucleotide differences were consistently detected when sequences of *Neospora* spp. isolates were compared to those of the *T. gondii* isolates. These results indicate that *Neospora* spp. and *T. gondii* are closely related, but distinct, species.

L5 ANSWER 12 OF 13 MEDLINE

DUPLICATE 3

ACCESSION NUMBER: 95097110 MEDLINE
 DOCUMENT NUMBER: 95097110 PubMed ID: 7799170
 TITLE: Phylogenetic relationship of *Sarcocystis neurona* to other members of the family Sarcocystidae based on small subunit ribosomal RNA gene sequence.
 AUTHOR: Fenger C K; Granstrom D E; Langemeier J L; Gajadhar A; Cothran G; Tramontin R R; Stamper S; Dubey J P
 CORPORATE SOURCE: Department of Veterinary Sciences, University of Kentucky, Lexington 40546.
 SOURCE: JOURNAL OF PARASITOLOGY, (1994 Dec) 80 (6) 966-75.
 Journal code: 7803124. ISSN: 0022-3395.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-U07812
 ENTRY MONTH: 199501
 ENTRY DATE: Entered STN: 19950215
 Last Updated on STN: 19950215
 Entered Medline: 19950125

AB *Sarcocystis neurona* is a coccidial parasite that causes a neurologic disease of horses in North and South America. The natural host species are not known and classification is based on ultrastructural analysis. The small subunit ribosomal RNA (SSURNA) gene of *S. neurona* was amplified using polymerase chain reaction techniques and sequenced by Sanger sequencing reactions. The sequence was compared with partial sequences of *S. muris*, *S. gigantea*, *S. tenella*, *S. cruzi*, *S. arieticanis*, *S. capracanis*, *Toxoplasma gondii*, *Eimeria tenella*, and *Cryptosporidium parvum*. Alignments of available sites for all 10 species and alignments of the entire SSURNA sequence of *S. neurona*, *S. muris*, *S. cruzi*, *T. gondii*, and *C. parvum* were performed. Alignments were analyzed using maximum parsimony and maximum likelihood methods to determine relative phylogeny of these organisms. These analyses confirmed placement of *S. neurona* in the genus *Sarcocystis* and suggested a close relationship to *S. muris*, *S. gigantea*, and *T. gondii*. Molecular phylogeny suggests that *Sarcocystis* spp., which utilize the dog (*Canis familiaris*) as the definitive host, evolved from a common ancestor, whereas those species (including *T. gondii*) that utilize the cat (*Felis domesticus*) as the definitive host evolved from another common ancestor. This suggests a possible definitive host for *S. neurona*.

L5 ANSWER 13 OF 13 MEDLINE
 ACCESSION NUMBER: 92292035 MEDLINE
 DOCUMENT NUMBER: 92292035 PubMed ID: 1818196
 TITLE: Identification and isolation of *Cryptosporidium parvum* genes encoding microtubule and microfilament proteins.
 AUTHOR: Nelson R G; Kim K; Gooze L; Petersen C; Gut J
 CORPORATE SOURCE: Parasitology Laboratory, Department of Medicine, San Francisco General Hospital, University of California 94143.
 CONTRACT NUMBER: AI07988 (NIAID)
 AI29882 (NIAID)
 AI29886 (NIAID)
 +
 SOURCE: JOURNAL OF PROTOZOOLOGY, (1991 Nov-Dec) 38 (6) 52S-55S.
 Journal code: 2985197R. ISSN: 0022-3921.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199207

ENTRY DATE:

Entered STN: 19920724

Last Updated on STN: 19970203

Entered Medline: 19920714

AB Microtubules and microfilaments are highly conserved cytoskeletal polymers hypothesized to play essential biomechanical roles in the unusual gliding motility of Apicomplexan zoites and in their invasion of, and development within, host epithelial cells. We have identified and isolated *Cryptosporidium parvum* genes encoding the microtubule proteins alpha- and beta-tubulin and the microfilament protein actin by screening a lambda gt11 *C. parvum* genomic DNA library with degenerate oligonucleotide and heterologous cDNA hybridization probes respectively. The alpha- and beta-tubulin genes have been partially sequenced and the deduced peptide sequences show greatest homology with the tubulins of the related parasites, *T. gondii* and *P. falciparum*. The complete nucleic acid sequence of the actin gene predicts a 376 amino acid, 42 kDa protein having 85% sequence identity with the *P. falciparum* actin I and the human gamma-actin proteins. Each of these cytoskeletal protein genes was demonstrated to be of cryptosporidial origin by Southern analyses of *C. parvum* chromosomes fractionated by pulsed field gel electrophoresis; the cloned alpha- and beta-tubulin genes hybridized with chromosomes of ca. 1,200 and 1,500 kb respectively and the cloned actin gene also hybridized with a 1,200 kb chromosome.

=>

L7 ANSWER 1 OF 3 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 2001669065 MEDLINE

DOCUMENT NUMBER: 21571769 PubMed ID: 11714523

TITLE: Real-time PCR for the detection of *Cryptosporidium parvum*.

AUTHOR: Higgins J A; Fayer R; Trout J M; Xiao L; Lal A A; Kerby S; Jenkins M C

CORPORATE SOURCE: USDA-ARS, Rm. 202, Bldg. 173, 10300 Baltimore Blvd., Beltsville, MD 20705, USA.. jhiggins@anri.barc.usda.gov

SOURCE: JOURNAL OF MICROBIOLOGICAL METHODS, (2001 Dec) 47 (3) 323-37.
Journal code: 8306883. ISSN: 0167-7012.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200203

ENTRY DATE: Entered STN: 20011121
Last Updated on STN: 20020308
Entered Medline: 20020307

AB Real time, TaqMan PCR assays were developed for the Cp11 and 18S rRNA genes of the protozoan parasite *Cryptosporidium parvum*. The TaqMan probes were **specific** for the **genus** *Cryptosporidium*, but could not hybridize exclusively with human-infectious *C. parvum* species and genotypes. In conjunction with development of the TaqMan assays, two commercial kits, the Mo Bio UltraClean Soil DNA kit, and the Qiagen QIAamp DNA Stool kit, were evaluated for DNA extraction from calf diarrhea and manure, and potassium dichromate and formalin preserved human feces. Real-time quantitation was achieved with the diarrhea samples, but nested PCR was necessary to detect *C. parvum* DNA in manure and human feces. Ileal tissues were obtained from calves at 3, 7, and 14 days post-infection, and DNA extracted and assayed. Nested PCR detected *C. parvum* DNA in the 7-day post-infection sample, but neither of the other time point samples were positive. These results indicate that real-time quantitation of *C. parvum* DNA, extracted using the commercial kits, is feasible on diarrheic feces, with large numbers of oocysts and small concentrations of PCR inhibitor(s). For samples with few oocysts and high concentrations of PCR inhibitor(s), such as manure, nested PCR is necessary for detection.

L7 ANSWER 2 OF 3 MEDLINE DUPLICATE 2

ACCESSION NUMBER: 2000429837 MEDLINE

DOCUMENT NUMBER: 20419416 PubMed ID: 10966225

TITLE: Detection and speciation of *Cryptosporidium* spp. in environmental water samples by immunomagnetic separation, PCR and endonuclease restriction.

AUTHOR: Lowery C J; Moore J E; Millar B C; Burke D P; McCorry K A; Crothers E; Dooley J S

CORPORATE SOURCE: Department of Applied Biological and Chemical Sciences, University of Ulster, Coleraine, Northern Ireland.

SOURCE: JOURNAL OF MEDICAL MICROBIOLOGY, (2000 Sep) 49 (9) 779-85.
Journal code: 0224131. ISSN: 0022-2615.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200009

ENTRY DATE: Entered STN: 20000922
Last Updated on STN: 20000922
Entered Medline: 20000908

AB Current methods for the detection of *Cryptosporidium* oocysts in water samples are both time-consuming and subject to variation in sensitivity. A **genus-specific** PCR assay was designed for the

specific amplification of a 552-bp region of the **18S** rRNA gene. Postamplification endonuclease restriction generated unique digest patterns that enabled differentiation between the three species, *C. muris*, *C. baileyi* and *C. parvum*, the major human pathogen. Theoretical restriction profiles for other *Cryptosporidium* species were also predicted. The assay routinely detected 10 oocysts in 10-ml purified oocyst preparations, but sensitivity was found to be 10(3)-10(4) -fold lower in environmental water samples. The use of Chelex resin and an immunomagnetic separation procedure overcame this inhibition. This provided detection levels of 10(1)-10(3) oocysts, depending on water turbidity. Rapid and sensitive pathogen detection methods are essential for the water industry. The results of this study demonstrate that PCR has the potential to improve current detection capabilities greatly by differentiating the major human pathogens from non-pathogenic species. This will greatly facilitate a closer examination of the epidemiology of this important pathogen.

L7 ANSWER 3 OF 3 MEDLINE DUPLICATE 3
 ACCESSION NUMBER: 97133950 MEDLINE
 DOCUMENT NUMBER: 97133950 PubMed ID: 8979344
 TITLE: Comparison of primers and optimization of PCR conditions for detection of *Cryptosporidium parvum* and *Giardia lamblia* in water.
 AUTHOR: Rochelle P A; De Leon R; Stewart M H; Wolfe R L
 CORPORATE SOURCE: Water Quality Laboratory, Metropolitan Water District of Southern California, La Verne 91750-3399, USA..
 SOURCE: prochelle@mwd.dst.ca.us
 SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1997 Jan) 63 (1) 106-14.
 Journal code: 7605801. ISSN: 0099-2240.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199703
 ENTRY DATE: Entered STN: 19970327
 Last Updated on STN: 19970327
 Entered Medline: 19970318
 AB Eight pairs of published PCR primers were evaluated for the specific detection of *Cryptosporidium parvum* and *Giardia lamblia* in water. Detection sensitivities ranged from 1 to 10 oocysts or cysts for purified preparations and 5 to 50 oocysts or cysts for seeded environmental water samples. Maximum sensitivity was achieved with two successive rounds of amplification and hybridization, with oligonucleotide probes detected by chemiluminescence. Primer annealing temperatures and MgCl₂ concentrations were optimized, and the specificities of the primer pairs were determined with closely related species. Some of the primers were species **specific**, while others were only **genus specific**. Multiplex PCR for the simultaneous detection of *Cryptosporidium* and *Giardia* was demonstrated with primers amplifying 256- and 163-bp products from the **18S** rRNA gene of *Cryptosporidium* and the heat shock protein gene of *Giardia*, respectively. The results demonstrate the potential utility of PCR for the detection of pathogenic protozoa in water but emphasize the necessity of continued development.

=>